Transcript for the Continuous Module of the on-line BMD Models Training

Slide 1. Welcome to the online Benchmark Dose Models training session for continuous BMD models.

Slide 2. The online benchmark dose training consists of five training modules. An introduction to dose response modeling, dichotomous models, a cancer model, continuous models, and nested models. In this session, we are going to discuss the benchmark dose modeling for continuous data.

Slide 3. As we mentioned in the introduction session, the purpose of the benchmark dose (BMD) modeling is to derive a point of departure for calculating a risk value, such as: a reference dose or a reference concentration. These values are calculated by dividing a point of departure by uncertainty factors. This point of departure can be the BMDL, which stands for the lower confidence limit on the benchmark dose estimate, or the BMCL which stands for the lower confidence limit on the benchmark concentration estimate.

Slide 4. Dose response analysis will assist in the identification of the most sensitive adverse effect. This most sensitive effect is also called the critical effect, and it is also used as the point of departure to use in establishing a toxicity benchmark. In the traditional approach, the point of departure is the no-observed adverse effect-level or NOAEL or the Lowest-Observed-Adverse-Effect-Level, LOAEL. But here, we are going to talk about another method of establishing the Point of Departure- that is the benchmark dose method, specifically the continuous benchmark dose module. Benchmark dose methods involve analyzing each endpoint's dose response separately.

In any toxicological experiment, various end points or observations can be monitored. Here we show some examples of common toxicological endpoints.
In this case, we monitor the clinical signs of toxicity, animal body weight, serum biochemical changes such as serum GPT levels, and liver pathological changes. The clinical signs such as convulsion and tissue pathological changes such as fatty liver are usually expressed as a yes or no response, or percent of animals having the effect. This kind of data are called dichotomous or quantal data. In contrast, the body weight and serum enzyme levels are usually expressed as continuum. Therefore, each animal will have an exact measurement of a value, and the group data are usually expressed as mean and standard deviation. These data are called continuous data.

Slide 5. Some examples of continuous effects are, body weight, organ weight, and serum enzyme levels. For each treatment group, the data are usually expressed as the mean +/- standard deviation or standard error. Body weights and organ weights are given as measurements not like categorical measurements. Serum enzyme level changes, for example SGPT, indicates some amount of liver damage and is measured in international units.
A complete data set should report the mean response, standard deviation and sample size. Please note that some times, you will also see data with standard error or SE. Because

the current BMDS software only takes standard deviation, the standard error needs to be converted. The standard deviation is equal to the standard error multiplied by the square root of the sample size.

Slide 6. This is an example of a modeled dose response curve for a continuous response. In the dichotomous dose response curve, the y-axis is expressed as a percent of animal response in each dose group. In the continuous dose response curve, the y-axis becomes actual measurement of the particular toxic response. For example if the toxic response is liver enzyme change, the y-axis unit becomes a continuous measurement, such as international enzyme unit. The x-axis would be the dose. In this BMD modeling curve, the actual data points or mean values appear as the green dots with error bars showing the upper and lower standard deviation.

Slide 7. The current benchmark dose software provided by EPA contains three types of statistical models for continuous data. The first one is the polynomial model, which is an all purpose model. The simplest model form is a linear model.
The second one is the power model. It usually shows an L-shaped dose response. Similar to the polynomial model, when the power term is set to one, the model becomes a linear model.
The third one is the Hill model, which shows a sigmoidal dose-response that plateaus. Because the Hill model is consistent with the receptor-mediated response, if the mode of action for a toxicity response is due to a receptor binding mechanism, the Hill model should be a preferred model.
In addition to these three models, you will also see a linear model function. If you want specifically to model the data with a linear model, you can use that. But keep this in mind, both the polynomial and power models can also generate a linear response model fit if the data indicate so.

Most of the time, we will use all three models to model the data, and then evaluate the results to select the most appropriate model.

Slide 8. To conduct benchmark dose modeling for continuous data, we will use the same procedure as we used for the regular dichotomous data.

First, we need to determine whether the data we have are worth modeling. Here, the same evaluation criteria we used for the NOAEL approach also apply.
For example, in order to do a good dose-response analysis, you need a quality study. In addition, you need to pay attention to the experimental conditions they used to generate these data. Ideally, the experiment should by conducted for an appropriate duration and through an appropriate route of exposure. If you are going to develop an oral risk value, ideally you should use oral exposure data. If it is an inhalation study you need to keep in mind the exposure duration. If you are going to develop a value for chronic exposure, you better have a chronic exposure study. You also need to pay attention to the organ or tissue where the response was observed. That means the study should measure the endpoints of concern. If you know the target organ for a particular chemical toxicity, but

a study did not evaluate the effects at that target organ or tissue, this study might be of limited value because it did not provide dose response data at the target organ, even though it did measure the effects at other organs or tissues.

Once you make your judgment about the dataset, ideally you are supposed to model all the endpoints or adverse effects possible. Once you have modeled all endpoints, then you can determine which response is the most sensitive based on the dose-response information.

There might be multiple effects in one study. The first adverse effect observed when the treatment dose increases, will be considered the critical effect, and it will be used as the point of departure to calculate the risk value.

Slide 9. For different end points, you might need different uncertainty factors. Therefore, the resulting reference dose or RfD or reference concentration or RfC will depend on the final product of the calculation as shown in this equation. Thus, some times, only conducting BMD modeling for the apparently most sensitive response might not capture the most conservative estimate when considering the uncertainty within the database.

Slide 10. Secondly, the data should contain a significant dose-related trend. If the data do not provide a dose-response change, then the model will not be meaningful.

In addition, a dose-response may not be a linear response, and most likely, they are curved response. In order to generate a curve dose-response fitting, you at least need to have three data points. If you only have two data points, you will only be able to generate a straight line. When you do a curve fitting to two data points, you will always have a perfect fit by any model, but the curve fitting to two data points provides no meaningful results because the limited information prevents a meaningful curve fitting to the data.

Lastly, you also want that data set containing the data points that had a response close to the benchmark response you are going to use. When there is a data point close to this benchmark response or BMR, a good model fit to the data should also provide good data fit at the BMR range. That will also provide a reliable estimate of the benchmark dose or BMD, which corresponds to the BMR. If you have a data point that is far away from the BMR, the estimated BMD will be rely heavily on extrapolation based on the curve fitting. This extrapolation may produce some uncertainty in the estimated BMD.

Slide 11. This figure shows you the importance of having a data point close to the BMR. For this data set, we have five data points. The lowest treatment dose data point is very close to the BMR. Because there is a data point at this response level, when you fit this data set with different mathematic models, one model might fit this low dose data point better than the other models. Based on the evaluation of the model result which we are going to discuss later in this training, you will choose this model as the best fitting model to estimate the BMD or BMDL. If other models do not provide a good fitting at this data

point, you might not choose those models. However, without this data point, there is no information in the data here to force the model fitting to a certain response. Thus, the BMD estimation would vary depending on the model used. This is also called model dependent BMD. This will introduce the uncertainty in the estimation of BMD or BMDL.

Slide 12. You should model all biologically, and statistically significant responses if feasible. As a range to consider, you at least need to model all endpoints with a LOAEL that is less than 10 fold above the lowest LOAEL of the database.
In some cases, it may not be possible to obtain a curve that provides a good fit to the entire dose-response for one endpoint, particularly if the response plateaus at high doses. In such cases, the mathematic model might compromise the fitting at low dose range in order to maximize overall data fitting. Because we are interested in the response at the low dose where BMR is located, it is more important for the curve to fit the data in the low dose region than to fit the overall dose-response. Therefore, the high dose group may be dropped if that improves the data fit at the response close to the BMR. Dropping the high dose also makes sense from a biological perspective. Plateaus or other changes in the dose response at the high dose may reflect changes in toxicokinetics (such as saturation of metabolism) or changes in toxic response that are not relevant to lower doses.

Slide 13. Now we will go over the general procedure for BMD modeling, but will focus on parameters used in the continuous BMD model

Slide 14. This is an example of what the curve for a continuous BMD looks like. The x-axis is the dose and the y-axis is continuous response. The green dots on the graph are the actual data points. The purpose of the BMD modeling is to place the best fit line, which is the red line on the graph, as close as possible to the data points. Based on this model fitting, an estimation of the confidence interval will be determined, this is the blue line on the graph. Based on this information, the benchmark dose response can be identified and the corresponding BMD and BMDL can be estimated.

Slide 15. The BMD analysis of an endpoint involves six steps.

These steps are:
choose BMR;
Select a model, set model parameters and run the model;
Evaluate model results;
Complete model runs with all the available models;
Evaluate BMDL;
And Identify the best fit model.

Eventually, use the BMD or BMDL to calculate the risk value.

Slide 16. Select a benchmark response.

The benchmark response or BMR should be near the low end of the range of increased risks that can be detected by a bioassay. It is not advisable to pick a vary small BMR due to the lack of statistical power needed to detect such change.

Low BMRs can impart high model dependence. If the data does not provide a response close to the BMR, extrapolation to the BMR can be done, but different models will behave differently due to lack of restriction by the data point in this low response range. This will cause different BMD estimates from the different models for this BMR. The data can become model dependent indicating that the estimate is not as reliable as if you had selected a BMR close to a data point available.

Slide 17. For continuous data, a BMR is usually expressed as an increased mean response.

The BMR can be measured in several ways.

As shown here, it can be expressed as an increased mean response from the control mean. Here, m(0) stands for mean response value at control dose (0), and m(d) for mean response at exposure dose (d).
Another way to express the BMR is the fraction of change in the control mean. It is calculated by dividing the change in the mean response from the control by the background response. For example, if the mean liver enzyme level in the serum in the control group is 150 unit, a 100% BMR indicates response level of 300 units as it is calculated as (300-150) and then divided by 150 units of background response.

The other way to express the BMR is a change from the control relative to the standard deviation. It is calculated by dividing the change in the mean response from the control by the standard deviation in the control group. If the mean response in the control group is 150 enzyme unit and standard deviation is 10. Then the BMR of one standard deviation is 160.

The default BMR for continuous data recommended by EPA is one standard deviation.

Slide 18. This is a continuous type model run window from BMDS software. After you input the data and select the specific model, you can see what parameters are needed for the specific model run. Here is where the type of BMR should be selected for each continuous model.

Slide 19. These are types of BMR you can choose. Let's see how we should select them.

The first one is the relative deviation. The BMR for relative deviation is calculated as control mean $\pm$ a fraction of control mean. This fraction change from the control mean is benchmark response factor, or BMRF. For example, if the BMR should be a 20% increase in the mean response, you need to select the relative deviation as the type of BMR, and enter 0.2 as the BMR value. Please note that the value you enter as BMR

means different things for different types of BMR as shown in this slide. For the relative deviation BMR, the value means the fraction of change in the mean.

The second type is absolute deviation BMR. In this case, the BMR is calculated as control mean ± the value you enter in the BMR. Therefore, it is an absolute value change from the control mean. The value you enter would be a continuous value of change such as 150 units of liver enzyme change.

The third type is standard deviation BMR. The BMR value for this type is calculated as control mean ± a factor of standard deviation in the control group. For example, if we decide to use the default BMR of one standard deviation, we select this type of BMR, and enter 1.0 in the BMR. Now, the value you enter means a factor of change in the standard deviation.

The point type of BMR requires direct input of the BMR value for BMD estimation. Here, you need to provide a response level as the BMR regardless of background response, standard deviation, etc.

For the Hill model, you can also select an extra type of BMR. The calculation of the BMR is shown in the slide. In this calculation, you basically define the BMR as a fraction of change that could occur.

Slide 20. This slide shows a selection for the default one standard deviation from the control. To do that, we need to select the standard deviation type of BMR, and enter 1.0 as the BMR. Again, the value entered here might mean different things when other types of BMR are selected.

Slide 21. To choose the BMR to evaluate, first determine if there are any acceptable levels of change in the endpoint that is considered biologically significant. If there is, then that change is the BMR. One of the most common endpoints reviewed is body weight change. A 10% decrease in body weight is usually considered a biologically significant response. For example if the response you are modeling is body weight decrease, use the 10% change in the mean as the BMR and run the model. Then rerun the model using the default SD of 1, and compare the results of the two model runs.

Slide 22. Why do we use the one standard deviation as default BMR?

A study conducted by Kenny Crump in 1984 indicated that if we have a continuous dose response shown as a normal distribution, we can always use a cut off level to separate affected and nonaffected animals. Here we assume the high response level, the worse the response. When you move this cut off level within the distribution, you will change the percentage individuals which would be considered affected or not affected.

If we assume there is 1% background response in the control group, the cut off level can be found from the normal distribution of the control response data. If a treatment causes a response in a group of animals, the mean response will increase if the variation keep the

same. Thus, the distribution shifts up ward, and relative position of the cut off level in the distribution will be changed accordingly.

This study found that when we shift the distribution curve up ward by 1.1 standard deviation, the same cut off level we used before in order to find 1% background response would result in a 10% increase in the number of animals affected in the shifted distribution. That means if you increase the mean response by 1.1 standard deviation, you will cause 10% extra animals being affected.

This 10% extra response is consistent with the default 10% extra risk we recommended for dichotomous data modeling. Therefore, EPA recommends to use the one standard deviation as the default BMR for continuous data modeling if you could not define the BMR based on biological considerations.

Slide 23. Once the BMR is selected, it is time to begin step 2. That involves the selection of a model, setting of the parameters and running the model.

Slide 24. As we mentioned previously, the BMDS software provides three types of models for continuous data. The polynomial model, power model and Hill model. We usually need to run all of these models for each data set in order to determine which model provides the best fitting to the data.

Slide 25. The goal of BMD modeling is to find the model that fits the data best. Keep in mind that nonlinear models do not necessarily have a biological interpretation. The criteria for the best model selection will be based on whether each model can describe the data set.

Nevertheless, there is a biological basis for the Hill model. It represents a receptor mediated response. Therefore, if the mechanism of the toxicity response is receptor mediated response, the Hill model might be more appropriate. If this is the case, you should focus on the Hill model results.

If there is no information regarding the mechanism of action, or the information available does not indicate a particular model, the criteria for final model selection will be based solely on whether various models describe the data

Slide 26. After you select the appropriate model, it is time to set the parameters specific for the data set and the model.
There are two major parameter settings in the continuous models. They are parameter restriction, and variance modeling.
First, let’s talk about parameter restriction.

Slide 27. After you input the data, assign data columns, and select the model type, the BMDS software will lead you to a second window called type model run. This is the type model run window where you need to select parameters and enter the BMR. In this particular case, we are running the polynomial model.

Here you can see, besides the BMR, we also need to decide whether we want to restrict the parameters, select the degree of polynomial, and whether we want to model the data with a constant variance.

Slide 28. Here is the equation for the polynomial model.
For this model, the more degrees of the polynomial you allow, the more beta terms will be introduced. If the degree of polynomial is set to one, it will be a linear model. Please note that the number of parameters allowed in the model is limited by the degrees of freedom or data set. The number of parameters should not be more than the number of data points in each data set. Thus, for a data set with 4 data points, the degree of polynomial should not exceed 3. Usually, we set the degree of poly to 2 or 3 unless more curvature is needed in the curve.

In addition to the degree of polynomial, we can restrict the coefficients to a specific sign. Some times, unrestricted polynomial coefficients may result in a wavy curve.

Slide 29. This polynomial example shows a wavy curve due to unrestricted betas. If we evaluate the model results without visual inspection, the model goodness of fit p value will indicate a perfect fitting because of the estimated curve going through all the data points. However, the visual inspection of the curve indicated a unrealistic fitting to the data. In this case, a beta restriction is needed. For an increased response, the beta should be restricted to non-negative.

Slide 30. This slide provides the equation for the power model. The power model contains three parameters: gamma, alpha, and beta. The BMDS allows a restriction on power larger or equal to one. If power goes to 1, it becomes a linear model. If a non-restricted power is used, a power of less than one may result in an infinite slope at the control dose.

Slide 31. This is an example of a power model without restriction. The slope of the curve at the control level is infinite which gives a supralinear response. In this case, when we use 1 standard deviation as BMR, it will provide an estimated BMDL of zero. This is not a biological response you should see. To solve this problem, we need to restrict the power.

Slide 32. This is the Hill model used in the BMDS software. The Hill model uses four parameters. Among these parameters, the power term can be restricted in the BMD modeling. Similar to the power model, unrestricted power may result in an infinite slope at the control dose. If this is the case, a restricted power is recommended.

Slide 33. The Hill Model provides a typical s-shaped curve. This model allows the calculation of an extra BMR based on maximum response level within the data set.

Slide 34. The Hill model can also show a response shown in this form without the initial threshold response. In this case, a non-restricted power in the model run may result in an

infinite slope at the beginning of the dose response curve. That is why we also have a choice of whether to restrict the power for the Hill model.

As a rule of thumb,when using continuous BMD models, we recommend to use restricted parameters when you run any of these models at the first time. Then, based on the model output, you can decide whether you want to run the model with other settings.

Slide 35. The second parameter setting is variance modeling. Continuous data is usually presented as mean +/- standard deviation. Therefore, in the continuous BMD models, measured data always have a mean and a standard deviation or standard error, In some cases, the variance in the data set does not change when the dose increases. While in other cases, the variance at each data points varies with the change in the dose. The BMD needs not only to describe the mean response, but also to describe the variance at each data point. Thus, the BMD models allow to either model the data with a constant variance or a modeled variance as shown in this slide.

In the current version of BMDS, the distribution of continuous measures is assumed to be normal, with either a constant variance or a variance that changes as a power function of the mean value. When constant variance is selected, the rho is set to zero, otherwise, the rho will be modeled as an extra parameter in the model. The BMDS software provides test results to assist the selection of this parameter. The Test 2 results in the BMD output file suggest whether variance needs to be modeled or not. If the test 2 result suggests a non-constant variance in the data set, the Test 3 result will indicate whether the variance is modeled successfully with the power model as shown here.

Slide 36. The next two slides are some examples of output information from the continuous model runs.
In each output file, there are results from Test 1, Test 2, Test 3 and Test 4 as shown in this slide. The meaning of each of these tests is also shown in the output file. Here, the Test 2 and Test 3 are specific for variance modeling.

The test 2 tests whether variances are homogeneous, and the test 3 tests whether variance was adequately modeled with the power model. The significant P values for these tests are listed below. In this case, the p values for both test 2 and test 3 are larger than 0.1.

Slide 37. The BMD output also provides explanation of these tests. Since the test 2 p value is larger than 0.1, you cannot reject the hypothesis that the variances are homogeneous in the data set. Thus, you can use the constant variance as your choice in the final model run.

Slide 38. If the test 2 p value is less than 0.1, this would suggest non-homogeneous variances. You should set the model to non-constant variances. The test 3 will provide a test on whether the variances are successfully modeled. A p value larger than 0.1 as shown here indicates that the modeled variance appears to be appropriate.

Slide 39. After selecting the model type and setting parameters, you can proceed with a model run. Here we are going to briefly summarize these procedures.

Slide 40. To run the model, you first must input the data either manually, input electronically from spreadsheet or copy and paste the information from a spreadsheet. If you copy data from a spreadsheet, you must close the spreadsheet program before you paste them in the BMD dataset screen. Once the data are entered, select continuous model from the model type, and then select a specific model you want to use. Then, you need to assign the data columns to each required data entry such as dose, sample size, mean and standard deviation. To do so, you need to select the name of the data column used in the data table from a corresponding pull down menu for each required data entry. After this is completed, proceed to next window.

Slide 41. The next pop up window is the "continuous type model run" window. This window is model specific and it is where you set the parameters for each particular model. In this case, you set the BMR, parameter restriction, degree of poly and variance modeling options. After setting all these parameters, if you want the BMDS software to estimate BMD and BMDL, and plot the BMDL curve line, you need also check the BMD calculation box and BMDL curve calc. box. Then you can start the model run by clicking the "run" button. After the model run is finished, the BMDS will provide an output sheet and a graph for model evaluation.

Slide 42. After each BMD model run, we need to evaluate the output results and BMD model fitting curve to determine whether the selected model fits the data, and how well it is fitting the data.

Slide 43. The goal of BMD modeling is to fit mathematical models to the data set in order to estimate a benchmark dose. Because the benchmark response is usually located in the low dose range, we need the model to fit the data, especially at the lower end of the observable dose-response range. Ideally, we need a model that will fit all the data points. But some times, the data are in such a way that the BMD models could not model all the data points very well. To maximize the overall fit to the data, the model some times might sacrifice the fit in the low dose range. This lack of good fit to the low dose range can be evaluated by visual inspection of the curve and evaluation of the BMD output results.

Slide 44. Evaluation of the BMD models takes into account the goodness of fit and a model comparison. There are three ways to evaluate the model fitting to the data: overall data fit or global measurement, visual inspection of the model fit, and the local measurement.

Slide 45. Global fitting measurement uses likelihood ratio test or deviance test which is included in the BMD software. The deviance between the ideally best fitted model, and the current fitted model is calculated as shown in this equation, and it follows chi square distribution. Therefore, the probability of this calculated deviance can be estimated, and used to evaluate the overall fit of the data.

Slide 46. In the output sheet, test 4 shows the deviance test result. In this example, the goodness of fit p value from the test 4 is 0.9466. As indicated in the result explanation, the model chosen seems to adequately describe the data because the P value is larger than 0.1. A p value of greater than 0.1 is considered acceptable model fit to the data.

Slide 47. Now, let's discuss the visual inspection of model fit.

Slide 48. A visual inspection of the model fit must also be done prior to accepting the model. Here is an example of a curve fitting figure. We need to review the graph to ensure the curve goes through or close to each data point, and pay close attention to the fitting at the low dose range where the BMR is to see how the curve fits the data point in this range.

In addition, we also need to evaluate whether the overall curve fitting is consistent with the toxicity response. Any unrealistic curve may also indicate some problem with the model.

Slide 49. Now let's see how to use the output result to evaluate the local model fit to the data, especially at the response range close to the BMR.

Slide 50. The BMD output file also provides a measurement for the local fitting to each data point called scaled residual. It is calculated based on distance between the data point and the corresponding estimated value by the model as shown in this equation. An absolute value of a scaled residual larger than 2.0 is considered poor fit to the data point.

Slide 51. This shows the "table of data and estimated values" found in the BMD output sheet. The scaled residuals for each data point are listed in the last column in this table. As we mentioned previously, we need to identify the scaled residual for the data point that has the response closest to the BMR we defined. As shown in the curve fitting figure in the previous slide, the data point at exposure dose level of 8 mg/kg has the response closest to the BMR of 1.0 standard deviation from the control mean. The scaled residual for this data point is 0.0771. This value is very small, and it indicates that the estimated response is very close to the actual data point.

Slide 52. Once we conclude that the model provides a satisfactory fit to the data, we can document the estimated BMD and BMDL provided by the model in the output sheet. At the end of each output sheet, you can find the BMD and BMDL for the BMR you specified. The output sheet also lists the risk type, the benchmark response, and the confidence level you specified in the model parameter setting section. In this case, it shows that the BMR was set to 1.0 standard deviation from the control mean, the confidence level was set to 95%, and the estimated BMD and BMD lower confidence limit are 12.2 and 9.2 mg/kg respectively.

Slide 53. After we run all the available models to fit the data, and identify the models providing acceptable fit to the data, we need to decide which model or models should be used to provide the final BMD or BMDL used as the point of departure.

To do so, first you need to compare the BMDLs obtained from these models. If the estimated BMDLs differ by more than 3-fold, this indicates a significant model dependence in BMD estimation. Because these BMDs or BMDLs are all obtained from acceptable models, and there is no specific reason to discard any of the models, the lowest BMDL will be used as the conservative point of departure to calculate risk values, such as an RfD or RfC.

If the BMDL values are within 3 fold, we need to decide which model fits the data better by comparing Akaike's Information Criterion or so called AIC value.

Slide 54. Here we will show you how the AIC value is calculated.

Slide 55. The AIC is used for model comparison because it not only considers the over all model fitting, but also considers the number of parameters used in the model. As we know in the curve fitting, more parameters in a model will provide more flexibility to the model. Therefore, introducing extra parameters should improve the model fitting to the data. If additional parameters do not provide a better model fitting, it is recommended to keep the model as simple as possible.

The AIC is calculated by -2 times log likelihood plus 2 times the number of parameters in the model.
Thus, the better the model fits the data, the smaller is the AIC. In contrast, the more parameters used in the model, the larger the AIC would be.
The smaller the AIC value, the better.

Slide 56. The BMD model output sheet provides the AIC values in the likelihoods of interest table. There are several AIC values listed in this table. The AIC value we are using for model comparison should be the one for the fitted model. In this example, we will use the AIC of 41.57, and compare this AIC with AICs from other models to find the best model

Slide 57. If AIC values among all the acceptable models are identical, and there is no way to distinguish a better model from the others, it is recommended to use an averaged BMDL from the models as the point of departure in the risk value calculation.

If one model provides a AIC lower than those from other models, the estimated BMDL from this model will be used as the point of departure.

Slide 58. Now I will show you an example of running a continuous model for a sample data set. You can start the BMDS software and run the model with the provided data while viewing this training presentation simultaneously, and compare your model results with the results presented here.

Slide 59. The example data we are going to use are shown in this table. Here we assume the data are liver weight measurement from a chronic mouse study conducted by a contract laboratory. The study was conducted following good laboratory practice. There are a total of 6 treatment dose groups which are control, 35, 105, 316, 948 and 2634 mg/kg body weight. Each dose group has 10 mice, and the data are reported as mean and standard deviation.

Based on the available information on the study, the data were from a good quality study, and they contain clear dose response trend with more than 2 treatment dose groups besides the control group. We decided to model the data with the continuous models within the BMDS software.

Slide 60. First, let's set the BMR for this continuous data modeling.
As we mentioned in previous slides, if there is an accepted level of change in the endpoint that is considered to be biologically significant, then that amount of change can be used as the BMR.
In the absence of information regarding the level of response to consider adverse, a change in the mean equal to one control standard deviation or (1.0 SD) from the control mean can be used.

Slide 61. For the sample data, because there is no biological basis for selecting a BMR suitable for the liver weight changes in mice, we will select the default 1.0 standard deviation as the BMR as recommended by EPA.

Slide 62. After you start the BMDS software, you can select to enter the dataset screen. You can enter the data manually, input from spreadsheet or copy and paste into this dataset screen.

You can change the column title by right click the title name, and then edit the title name.

Once you finish entering the data, select continuous model from the model type pull down menu and select a specific model from the model pull down menu.

Next, we need to assign data to dose, subjects in dose groups, mean and standard deviation by selecting corresponding column titles from pull down menus.

For this example, we choose to run the Hill model.
For data assignment, we assigned dose column to dose, N column to number of subjects in dose group, mean column to "mean", and SD column to standard deviation.

Once we finished these selections, we can proceed to next window.

Slide 63. In this type model run window, we need to provide the parameters for the Hill model.

For this example, we selected standard deviation as the BMR type, and entered 1 for BMR to indicate a setting for 1.0 SD from the control mean.

For continuous model run, we always have a choice of using constant variance or non-constant variance. Here we select the constant variance to do a prerun, and we might adjust the selection for the variance based on the prerun results.

We selected the restricted power as recommended, and checked BMD calculation to request estimated BMD and BMDL from the output sheet. The default confidence limit is left to the 0.95 default value.

After all the parameters are selected, we can start the model by clicking the run button.

Slide 64. After the BMDS finishes the model run, you will see two pop up windows, one for curve fitting figure, and the other for the output sheet.

The visual inspection of this figure indicates that the model provides a good fitting to the high dose data points, but poor fitting for the low dose data points, especially at the BMR range as pointed out here.

Slide 65. From the output sheet, we can see the goodness of fit P value from test 4 is less than 0.0001. Thus, this model did not provide a good overall fitting to the sample data. Based on our visual inspection, the major issue with this model run is the relative good fitting at the high dose range and poor fitting at the low dose range. As we know, our emphasis is good model fit in the low dose range. Therefore, we can try to drop off the highest dose to see if this will improve the model fitting to the data.

Slide 66. This is the curve fit figure from the model run after the highest dose is dropped. Comparing to the previous model run, this run improved model fit for the low dose range, but the estimated value (the red line) is still a little away from the low dose data points. The goodness of fit P value is still less than 0.1, the minimum requirement for model acceptance.
We decided to further drop the second highest dose, and run the model again.

Slide 67. This is the model run results after the 2nd highest dose is dropped. In this case, the model now fits all the data points very well, especially for the low dose data points. The goodness of fit p value is 0.9341, larger than the required 0.1. The p-value increased significantly compared to the previous 2 model runs, resulting in an overall improvement in the curve fitting for low dose data points.

Slide 68. From the output sheet, we can find not only the goodness of fit p value, but also the AIC and test result for constant variance. In this case, the test 2 for the constant variance indicates that a homogeneous variance model appears to be appropriate.

Slide 69. We summarized the model run results in this summary table.

We can see that the Hill model could not provide a satisfactory model fitting to 6 and 5 data sets. The goodness of fit P values are much less than the required 0.1. After removing the two highest doses, the goodness of fit P value increased to 0.9341, and scaled residual at the data point closest to BMR decreased from 2.3 to 0.037. the small scaled residual value suggests a very good fit at the low dose range.

Please note that in the first model run with the full data set, the test 2 for the constant variance resulted in a p value less than 0.1. Therefore, the model was rerun to allow modeled variance with a rho value of 3.138. In contrast, the model run for 5 and 4 data sets indicated a homogeneous variance in the data sets. Thus, we used a setting of constant variance in these latter model runs, and rho value was set to zero.

Slide 70. Based on the comparison, removing the two high doses will result in a better model fit at the range of BMR. The estimated BMD and BMDL from the 4 dose data set are considered appropriated.

Slide 71. We also need to model the data with other BMD models. Here we will show you the results.

Slide 72. Based on our model runs with the Hill model, we only obtained satisfactory model fit to the data set without the two highest dose groups. Therefore, we only modeled the 4 dose data set with the other two BMD models. They are the polynomial model and power model.

Here is the curve fit figure produced by the polynomial model with no restriction to the beta. Our visual inspection indicates that the unrestricted model provided a bimodal response curve which is not consistent with normal toxicity response. Thus, we rerun the model with the betas restricted to non-negative.

Slide 73. For the same data set, restricting the beta resulted in a linear dose response. This model does not provide good fitting to the data as shown in this figure and low goodness of fit p-value of less than 0.0001.

So, the polynomial model did not provide good fit to the data.

Slide 74. Here is the model results with the Power model using non-restricted power. Although, the goodness of fit p-value for this model was 0.47, but our visual inspection of the curve fitting indicates that there is a supra-linear situation at the control dose which resulted in a very low estimated BMD and BMDL of zero. This is not biologically meaningful. Thus, we decided to restrict the power term.

Slide 75. As shown in this figure, the restricted power resulted in a straight line just as the restricted polynomial model, and a goodness of p value of less than 0.0001. Thus, the power model could not provide good fitting to the data either.

Slide 76. We exclude the Polynomial model results because the Non-restricted model resulted in a wavy curve, and the Restricted model provided a poor data fit.
We also exclude the Power model results because the Non-restricted model resulted in an infinite slope at the control dose, and the Restricted model provided a poor data fit to the data.

Slide 77. Therefore, in our model runs, the Hill model was the only model that provided a good data fit.

Slide 78. These are the summary results for the Hill model as we showed previously. We are going to used the estimated BMD and BMDL from the 4 dose data set as highlighted.

For this data set, the final BMD is 12.3 mg/kg and the BMDL is 5.8 mg/kg.

Slide 79. Let's summarize our continuous BMD modeling for the sample data:
How a model fits the data can be determined by visual inspection and the p value where the goodness of fit p value should be larger than or equal to 0.1.
The statistical and visual comparison among different continuous models indicated that the Hill model provides the best model fit to the data.
Comparison across models for the data set containing different dose groups using visual inspection, AIC and scaled residuals indicates that removing the two highest doses will result in a significantly improved model fit to the data, especially at the low dose response region.

Slide 80. For the variance setting, whether to use constant variance can be based on test 2 results from a pre-model-run. When both constant and non-constant variance settings resulted in satisfactory model fits, use the constant variance so that less parameters will be used in the final model.

Slide 81. This concludes our training module on continuous BMD models.